parent (0) and 10-1 as the paternal parent. The results are very similar to that seen in the 10-1 selfed photograph.

(c) W38 Control and 10-1 x 0 - W38 as previously described. 10-1 x 0 represents 10-1 as the maternal parent and W38 as the paternal parent (0). The results are very similar to that seen in the 10-1 selfed photograph except that in this photo segregation of the herbicide resistance trait can be seen in the 10-1 progeny.

Figure 3 illustrates the effect of Pursuit® postemergent application in tobacco seedling growth, as measured by plant height. C" represents the mean of the control (susceptible parent cultivar) plants, while each subsequent bar represents an individual transgenic progeny.

Figure 4 illustrates the effect of the imidazolinone herbicide Pursuit® on AHAS enzymes derived from an E. coli transformed with a Trp574 deletion [DEL], a Trp574 substitution [SUB] and an E. coli transformed with wild-type [WT] AHAS sequence.

Figure 5 illustrates the effect of the sulfonylurea herbicide Glean® on AHAS enzymes extracted from E. coli transformants. Abbreviations as in Figure 4.

Figure 6 illustrates the effect of the imidazolinone herbicide Pursuit® on AHAS enzymes extracted from E. coli transformants. The enzymes are derived from an E. coli transformed with a Ser653 deletion [DEL], an E. coli transformed with a Ser653 substitution [SUB], and an E. coli transformed with a wild-type [WT] AHAS sequence.

Figure 7 illustrates the effect of the sulfonylurea herbicide Glean® on AHAS enzymes extracted from E. coli transformants. Abbreviations as in Figure 6.

Figure 8 illustrates the effect of the imidazolinone herbicide Pursuit® on an AHAS enzyme derived from a tobacco plant with an Agrobacterium strain containing a mutant Arabidopsis AHAS allele having the Trp574 deletion.

DETAILED DESCRIPTION OF THE INVENTION

The entire nucleotide sequence of the AHAS gene in a number of organisms including yeast, E. coli, Brassica, Arabidopsis, sugar beet and tobacco has been previously disclosed (Mazur et al., supra. Lee et al., EMBO J. 7:1241, 1988). Moreover, it has been noted that herbicide resistance is associated with one or more mutations within the AHAS sequence; specifically, resistance has been noted to be associated with certain specific amino acid substitutions within the sequence. (EP 258 793; Yadav et al. PNAS USA 83:4418-22, 1986; Sathasivan et al., Nucl. Acids res. 18: 2188, 1990). It has, however, been implied (Hartnett et al., in Managing Resistance To Agrichemicals, Ch. 31, pp. 459-473, American Chemical Society, 1990; EP 258 793) that these regions of conservation in which mutations conferring resistance occur must generally be maintained to conserve enzymatic function.

It has now been unexpectedly discovered that conservation of these sequences is apparently not essential to the catalytic function of the molecule. In the course of development of the present invention, site directed mutagenesis of the Arabidopsis AHAS coding region is utilized to create deletion mutations in which a residue which has previously been shown to be substitutable is then deleted from the sequence. Deletion of a residue clearly destroys the conserved nature of the regions in which they occur. Nonetheless, the deletion mutations thus created all retain AHAS function, while also exhibiting herbicide resistance. Specifically, single deletions of the Trp574, Pro197 and Ser653 residues and double deletions of Pro197 and Ser653 are made to create fully functional, herbicide resistant plants. All the numbering of amino acids herein is based on the Arabidopsis AHAS sequence. However, it will be understood that, throughout the specification and claims, reference to a specific site of deletion in the Arabidopsis sequence is meant to encompass the corresponding sites in the AHAS sequence of any other species having an AHAS gene.

In view of these data, it becomes apparent that conservation of these herbicide-resistance associated regions of the AHAS molecule is not critical for catalytic activity, and that one or more amino acid deletions in one or more "conserved" amino acid sequences can easily be tolerated by the enzyme and further, will confer herbicide resistance to the plant. The present invention encompasses AHAS DNA sequences in which one or more deletions have been made relative to the wild-type sequence, which deletions do not alter the catalytic function of the resulting AHAS enzyme, but which confer herbicide resistance. Such deletion mutation include, but are not limited to, deletion of one or more codons, encoding the so-called "conserved subsequences" as defined in EP 257 783. These are DNA sequences encoding amino acids 119-122, 194-197, 201-208, 255-257, 348-353, 373-377, and 569-578 in Arabidopsis AHAS. Additionally, a further "conserved sequence" in which substitution has been described and in which deletion is useful in bringing about herbicide resistance, is 650-653. Deletion of one codon, more than one codon, and up to all the codons in the "subsequences" noted above, is considered within the scope of the invention. Preferred

are codon deletions encoding mutants having a deletion of at least one residue position selected from the group consisting of amino acid 120, 121, 197, 205, 256, 351, 376, 571, 574, 578 and 653. Particularly preferred are sequences encoding deletions at 197, 574 or 653.

Although the above-identified deletions represent regions in which variation is known to be tolerated, it is likely, in view of the substantial "flexibility" of the molecule that other deletions would also be feasible. For example, other apparently "conserved" regions of the AHAS enzyme also exist in addition to those outlined above. While not wishing to be bound by any particular theory, it now appears likely that, rather than being sites connected with catalytic activity of the molecule, and therefore requiring essential structural conservation, most of these conserved regions represent binding sites for the herbicide. Deletion of one or more amino acids at these sites would then logically prevent herbicide binding, and consequently, prevent herbicide interference with AHAS activity. Using this rationale, the available knowledge regarding "conserved" AHAS sequences (See, e.g., Mazur et al., Plant Physiol. 85: 1110-1117, 1987) and known techniques, such as site-specific mutagenesis, it would be a routine matter to design additional deletion mutants likely to have herbicide resistance. Putative mutants can then be screened by growth in the presence of inhibitory amounts of the herbicide of interest, to determine whether or not herbicide resistance has been conferred.

As with all proteins, the functional AHAS enzyme possesses a three-dimensional structure which is the eventual result of the linear arrangement of the component amino acids. Interaction of the side groups of amino acids produces a protein's secondary structure, and further folding results in a protein's tertiary structure. A given protein's specific "architecture" which ultimately results from the amino acid sequence can be critical to the protein's function. Thus, the substitution of an amino acid retains the overall structural integrity of the protein, whereas the deletion of an amino acid effectively destroys this structural integrity and can be expected to significantly alter the protein's three-dimensional structure and, in doing so, alter the function of the molecule. Therefore, in view of the potential damage that can be caused by a deletion, it is particularly surprising that the resultant molecule retains sufficient portions of its three dimensional structure to remain not only catalytically functional, but also to cause herbicide resistance.

Those skilled in the art will recognize that the deletion mutants of the present invention are not limited as to the source of the DNA or enzyme. Although the present experimental design primarily utilizes the Arabidopsis AHAS gene sequence, similar mutations can routinely be made in the AHAS sequence of any organism possessing such a gene, e.g. other higher plants, yeast, E. coli, and other microorganisms. The similarities in the wild-type AHAS gene among all the known sequences are so great as to render it a matter of routine experimentation to create identical mutants in AHAS sequences other than that of Arabidopsis. Alternately, chimaeric genes can be constructed which contain the deletion mutant portion of the Arabidopsis AHAS gene recombined with unaltered portions of the AHAS gene from other sources.

The novel gene types described herein may confer resistance to one, or more than one, type of herbicide. As has already been well established, AHAS is the site of action of several distinct classes of herbicides, namely, imidazolinones, sulfonylureas, triazolopyrimidines, sulfamoylureas and sulfonylcarboxamides. As with herbicide resistance conferred by amino acid substitution, the herbicide resistance conferred by such mutations may be selective to a particular herbicide, or there may be cross-resistance to more than one herbicide. For example, deletions of Trp574 and Ser653 create cross-resistance to both imidazolinones and sulfonylureas. One skilled in the art can, however, easily determine the specificity of any particular mutant by screening separately in the presence of, e.g., imidazolinones only, or sulfonylureas only, and isolating surviving plants. Cross resistance can be determined by growth in the presence of more than one class of herbicide. Types of herbicides with which the present invention is useful are described, for example, in U.S. Patent No. 4,188,487; 4,201,565; 4,221,586; 4,297,128; 4,554,013; 4,608,079; 4,638,068; 4,647,301; 4,650,514; 4,698,092; 4,701,206; 4,709,036; 4,752,323; 4,772,311; and 4,798,619; U.S. Pat. Nos. 4,127,405; 4,435,206; 4,424,703; 4,417,917; 4,398,939; 4,394,506; 4,391,627; 4,383,113; 4,378,991; 4,372,778; 4,371,391; 4,370,480; 4,370,479; 4,368,320 (sulfonylureas).

At this time, AHAS has been demonstrated to be not only present in a wide variety of plants, but has also been shown to be a critical site determining herbicide sensitivity in a broad range of essentially unrelated plants, e.g. corn, Brassica, tobacco, flax, Arabidopsis, and sugar beet (Sougaard et al., Mol. Gen. Genet. 219: 413-420, 1989; Jordan & McHughen, J. Plant Physiol. 131: 333-338, 1987; McHughen, Plant Cell Reports 8: 445-449, 1989). As noted above, then, it is possible to create the relevant mutation directly in the plant of interest by known mutagenic techniques. (See, for example, Manfatis, et. al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, 1982; and Example II, infra). However, it will frequently be more convenient to create transformed plants with a known and isolated DNA sequence comprising the requisite deletions, such as the Arabidopsis deletion mutants described in the present case. Plasmids containing deletion mutants at positions 574 and 653 have been deposited with the American

Type Culture Collection, Rockville, MD, on December 6, 1990 as, respectively, accession numbers ATCC 68488 and 68489.

Isolated AHAS DNA sequences of the present invention are useful to transform target crop plants, and thereby confer herbicide resistance without the necessity for mutagenesis. A broad range of techniques currently exist for achieving direct or indirect transformation of higher plants with exogenous DNA, and any method by which the novel sequence can be incorporated into the host genome, and stably inherited by its progeny, is contemplated by the present invention. A detailed description of one such method is provided in the following examples.

Indirect transformation of plant cells can be achieved by the use of vectors. A common method of achieving transformation is the use of Agrobacterium tumefaciens to introduce a foreign gene into the target plant cell. For example, in the present case, the mutant AHAS sequence is inserted into a plasmid vector containing the flanking sequences in the Ti-plasmid T-DNA. The plasmid is then transformed into E. coli. A triparental mating among this strain, an Agrobacterium strain containing a disarmed Ti-plasmid containing the virulence functions needed to effect transfer of the AHAS containing T-DNA sequences into the target plant chromosome, and a second E. coli strain containing a plasmid having sequences necessary to mobilize transfer of the AHAS construct from E. coli to Agrobacterium is carried out. A recombinant Agrobacterium strain, containing the necessary sequences for plant transformation is used to infect leaf discs. Discs are grown on selection media and successfully transformed regenerants are identified. The recovered plants are resistant to the effects of herbicide when grown in its presence. Other plant vectors, such as plant viruses, also provide a possible means for transfer of exogenous DNA.

Direct transformation of plant cells, instead of the use of vectors, can also be employed. Typically, protoplasts of the target plant are placed in culture in the presence of the DNA to be transformed, and an agent which promotes the uptake of DNA by protoplast is absorbed on their surfaces. Useful agents in this regard are polyethylene glycol or calcium phosphate.

Alternatively, DNA uptake can be stimulated by electroporation. In this method, an electrical pulse is used to open temporary pores in a protoplast cell membrane, and DNA in the surrounding solution is then drawn into the cell through the pores. Similarly, microinjection can be employed to deliver the DNA directly into a cell, and preferably directly into the nucleus of the cell.

In each of the foregoing techniques, transformation occurs in a plant cell in culture. Subsequent to the transformation event, plant cells must be regenerated to whole plants. Techniques for the regeneration of mature plants from callus or protoplast culture are now well known for a large number of different species (see, e.g., Handbook of Plant Cell Culture, Vols. 1-5, 1983-1989 McMillan, N.Y.) Thus, once transformation has been achieved, it is within the knowledge in the art to regenerate mature plants from the transformed plant cells.

Alternate methods are also now available which do not necessarily require the use of isolated cells, and therefore, regenerative techniques, to achieve transformation. These are generally referred to as "ballistic" or "particle acceleration" methods, in which DNA coated metal particles are propelled into plant cells by either a gunpowder charge (Klein et al., Nature 327: 70-73, 1987) or electrical discharge (EPO 270 356). In this manner, plant cells in culture or plant reproductive organs or cells, e.g. pollen, can be stably transformed with the DNA sequence of interest.

The present invention can be applied to transformation of virtually any type of plant, both monocot and dicot. Among the crop plants for which transformation to herbicide resistance is contemplated are corn, wheat, rice, millet, oat, barley, sorghum, alfalfa, sugar beet, Brassica species, tomato, pepper, soybean, tobacco, melon, squash, potato, peanut, pea, cotton, or cacao. The novel sequences may also be used to transform ornamental species, such as rose, and woody species, such as pine.

The novel sequences of the invention also is useful as selectable markers in plant genetics studies. For example, in plant transformation, sequences encoding herbicide resistance could be linked to a gene of interest which is to be used to transform a target herbicide - sensitive plant cell. The construct comprising both the gene of interest and the herbicide resistant sequence are introduced into the plant cell, and the plant cells are then grown in the presence of an inhibitory amount of the herbicide. Plant cells surviving such treatment presumably have acquired the resistance gene as well as the gene of interest, and therefore, putative transformants are readily identifiable.

The invention is further illustrated by the following non-limiting examples.

EXAMPLE I

Isolation of a Genomic Clone Encoding Arabidopsis AHAS

A 2.1Kb EcoRI fragment encompassing the promoter, transit peptide and a portion of the mature coding region of Arabidopsis AHAS is ^{32}P-labelled by nick translation (Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982) and utilized as a hybridization probe to screen a genomic library prepared from Arabidopsis thaliana genomic DNA (Clontech, Palo Alto, CA). The filters are hybridized for 24 hr. at 42°C in 6xSSC, 5 x Denhardt's solution, 50mM sodium phosphate, pH7.2, 0.1% SDS and 100ug/ml of denatured salmon sperm DNA. Filters are washed several times at 60°C in 1xSSC and 0.1%SDS. Six recombinant bacteriophage (A22, A31, A42, A52, A72, A83) were classified as putative positives. These bacteriophage are plaque purified and infected at low multiplicity with the E. coli strain K802 in liquid NZY broth (NZCYM is 10 g NZ amine, 5 g NaCl, 5 g yeast extract, 1 g casamino acids, 2 g $MgSO_4$-$7H_2O$ and 10ml of 1M Tris-HCl, pH7.2) as described by Maniatis, et al, supra. Liquid cultures are incubated overnight with constant shaking (300 rpm) at 37°C. The 50ml suspension is brought to 1M NaCl and 8% PEG by the addition of solid NaCl and solid polyethylene glycol. The suspension is incubated overnight at 4°C to precipitate the bacteriophage. The bacteriophage are pelleted via centrifugation at 18,000 x g for 20min. The pellet is resuspended in 2ml of TM buffer (10mM tris-HC1, pH 7.5; 10mM $MgC1_2$), layered upon a CsC1 step gradient (4.8M CsC1, 4.0M CsC1 and 3.2M CsCl) and centrifuged at 50,000 x g for 1 hr in a SW60 swinging bucket rotor. The phage band is removed from the 4.0/3.2M CsCl interface to a 1.5ml Eppendorf tube and the bacteriophage lysed by adding 1 volume of formamide and incubating at room temperature for 30 minutes. DNA is recovered by bringing the solution to 10m MTris-HC1, pH 8.0 and 1mM Na_2- EDTA and precipitating the DNA via the addition of 2 volumes of 100% ethanol. Bacteriophage DNA is recovered via centrifugation, washed with 70% ethanol and resuspended in TE buffer (TE buffer is 10mM Tris-HC1, pH 7.5; 1mM Na2-EDTA). The purified DNA is routinely extracted one to several times with phenol:chloroform:isoamyl alcohol (24:24:1), ethanol precipitated, and resuspended in 50-100ul of TE buffer prior to digestion with various restriction enzymes.

DNA preparations from the six recombinant phage are digested with various restriction enzymes and resolved by electrophoresis through a 1% agarose gel. The DNA is transferred to nitrocellulose (Soulhern) and hybridized (24 hr. at 60°C in 2xSSC, 5 x Denhardt's solution, 50mM sodium phosphate, pH7.2, 0.1% SDS and 100ug/ml of denatured salmon sperm DNA) against a radiolabeled 900 bp Nco1/EcoRI fragment representing the 3' region of the 2.1Kb EcoRI fragment (the transit peptide and the 5' portion of the mature coding region). The filter is washed twice at 65°C in 0.5x8SC and 0.1%SDS. The 900 bp Nco1/EcoRI fragment hybridizes to a 5.5Kb Xba1 and 2.1 EcoRI fragment of both A42 and A52. These data are in agreement with published information concerning the cloning and characterization of the genomic sequence encoding Arabidopsis AHAS (Mazur et al., Plant Physiol, 85: 1110-1117, 1987).

The bacteriophage A52 is chosen for further analysis. The 5.5 Kb Xbal fragment of A52 is isolated from a 1% agarose gel and purified through an IBI electroelution device as suggested by the manufacturer. The 5.5Kb Xba1 fragment is ethanol precipitated and resuspended in ddH_2O. These fragments are ligated to Xbal digested-pSK(-) plasmid DNA (purchased from Stratagene, La Jolla, CA). The fragments are ligated for 16-24 hours at 14°C in a 20ul volume as described by Maniatis, et. al., supra. Following incubation, the 20ul reaction volume is diluted to 100ul with 100mM Tris-HC1, pH7.2. Twenty five ul of this sample is diluted further to 100 ul with 100mM Tris-HC1, pH7.2 and incubated with 100ul of competent HB101 cells [prepared exactly as described by Morrison (Meth. Enzymol. 68: 326 - 331, 1979)]. The plasmid/E. coli transformation mix is incubated on ice for 20 minutes; transferred to 42°C for 2 minutes; room temperature for 10 minutes; followed immediately by the addition of 1 ml of LB broth (LB broth is 10 g bacto-tryptone; 5 g yeast extract; 5 g NaC1 and 10 ml 1 M Tris-HC1, pH 7.2 per liter) and further incubation at 37°C for 20 minutes. DNA plasmid cloning vehicles used in this work carry either the gene conferring resistance to ampicillin or kanamycin. Therefore, 200 ul of each transformation mix is plated directly onto either LB_{amp} or LB_{kan} plates (100 ug/ul of either respective antibiotic) and incubated at 37°C for 12-20 hr, depending on colony size.

Colonies are transferred to 3ml of LB_{amp} or LB_{kan} and incubated overnight at 37°C with constant shaking. Small scale plasmid DNA preparations are prepared by minor modifications of the alkaline lysis method described by Maniatis, et al. supra. Routinely, 400 ul of the 3ml ovemight culture is pelleted in a 1.5ml Eppendorf tube and resuspended in 150 ul of GTE solution (GTE solution is 50mM glucose; 25mM Tris-HC1, pH 8.0 and 10mM Na2-EDTA) and incubated on ice for 20 minutes, followed by the addition of 300 ul alkaline-SDS solution and 200 ul of 5M K^+/3M OAc at the prescribed time intervals. The final DNA pellet is resuspended in 40ul of 50ug/ul RNAse A in TE buffer. Fifteen ul of plasmid DNA is removed for restriction digestion. Two plasmids, pAC301 (5'—>3') and pAC302 (3'—>5'), represent constructions containing the 5.5Kb Xbal fragment ligated to the Xbal site of pSK(-). Further restriction analysis of pAC301 and pAC302 confirm the presence of the promoter region, transit peptide, mature coding region and 3'non-

translated regions of the genomic sequence encoding Arabidopsis AHAS (Mazur et al., Supra).

EXAMPLE II

Oligonucleotide site directed mutagenesis of the Arabidopsis AHAS coding region

(a) Preparation of single stranded template of pAC301 - The plasmid pAC301 is transformed into the E. coli strain, XL-1 (Stratagene). Single stranded template of pAC301 is generated from this pSK(-) "phagemid" based construction by following the manufacturers protocol. Routinely, a 3ml SB_{amp} overnight culture is added to a sterile flask containing 50ml of SB_{amp} (SB is 35 g bacto-tryptone, 20 g yeast extract, 5 g NaCl and 10 ml of 1M Tris-HCl, pH 7.5 per liter) and incubated at 37°C with constant shaking (300 rpm) until the culture reached an OD600 of 0.3. At this point the culture is inoculated with the helper phage, R408 (1×10^{10} phage) and vigorous 10" shaking at 300 rpm at 37°C was continued overnight. The bacteria are pelleted via centrifugation at 10,000 x g for 10 minutes. The bacterial pellet is discarded and the supernatant (approximately 45 ml) containing the single stranded pAC301 template is stored at 4°C until use. The supernatant is routinely used for up to one month for single stranded phagemid preparations. Small scale preparations (1.2ml) of single stranded pAC301 phagemid DNA are prepared essentially as described by the manufacturer (Stratagene). The phage are precipitated by adding 300 ul of a 3.5M NH_4Ac, pH 7.5; 20% PEG solution. The solution is mixed well in a 1.5 ml Eppendorf tube and incubated at room temperature for 20 minutes. The single stranded phage are pelleted by centrifugation for 20 minutes in an Eppendorf microcentrifuge. The supernatant is decanted, the pellet dried and eventually resuspended in 300 ul of TE buffer. The phage are lysed by adding 200 ul of phenol:chloroform:isoamyl alcohol (24:24:1) and vortexing for 1 minute. This step is repeated once more and followed by two more extractions with chloroform:isoamyl alcohol (24:1). The final aqueous phase is ethanol precipitated (one-tenth volume 5M K^+/CMOAC and two volumes of 100% ethanol), washed with 70% ethanol, resuspended in 20ul of TE buffer and transferred to a fresh 1.5ml Eppendorf tube.

(c) Preparation of the oligonucleotide - All oligonucleotides are purchased from New England Biolabs. Oligonucleotides are utilized to introduce (1) an amino acid substitution at the Trp574 residue of the Arabidopsis AHAS coding region (Trp --> Leu) and (2) an amino acid deletion at the Trp574 amino acid residue.

```
Trp574 --> Leu substitutions
                 V   N   Q   W   E   D   R
wild type 5'-GTT ATG CAA TGG GAA GAT CGG-3'
                 V   N   Q   L   E   D   R
mutant    5'-GTT ATG CAA TTG GAA GAT CGG-3'(21-mer)

Trp574 deletion
                 V   N   Q   W   E   D   R
wild type 5'-GTT ATG CAA TGG GAA GAT CGG-3'
                   V   N   Q       E   D   R
mutant    5'-G GTT ATG CAA --- GAA GAT CGC- 3'
                          (21-mer)
```

Two hundred nanograms of each oligonucleotide is subjected to a kinase exchange reaction prior to hybridization to pAC301 single stranded template. A 40 ul reaction volume included 50mM Tris-HCl, pH7.5; 10mM MgCl2, 50mM DTT, 0.1mM spermidine and 0.1mM Na2-EDTA. The reaction is initiated via the addition of 10 units of T4 polynucleotide kinase (Pharmacia) and is incubated at 37°C for 30 minutes. The reaction is terminated by bringing the reaction mixture to 90°C for 3 minutes. One-half of the kinase reaction is added to the entire 20 ul pAC301 single stranded prep and is incubated at 65°C for 10 minutes to promote hybridization. To this 40 ul kinase/oligonucleotide mix is added 6 ul of 1mM dNTP's, 6 ul of 10 x

ligase buffer (500mM Tris-HCl, pH7.5; 70 mM $MgCl_2$ and 10mM DTT), 2 ul 10mM rATP, 5 units of Klenow DNA polymerase (Pharmacia), 8 units of DNA ligase (Stratagene) and 4ul ddH_2O. The polymerase/ligation reaction is incubated at room temperature for 3 hr. One-half of the mix is used to transform competent E. coli XL-1 cells. The transformation mix is spread to LB_{amp} plates and incubated overnight at 37°C. Transformants are restreaked to fresh LB_{amp} plates in a grid fashion. Colony screening by hybridization was performed exactly as described by Stratagene Technical Service (June 1988/pBSII Exo/Mung Bean DNA Sequencing System). Both oligonucleotides are ^{32}P-labeled by a kinase exchange reaction (300 ng of oligonucleotide in the presence of 40 uCi of $\alpha^{32}P$-ATP utilizing kinase conditions described previously). Unincorporated ^{32}P-ATP is removed by electrophoresing the kinase reaction through a 20% acrylamide/7M urea gel. The radioactive band corresponding to the 21-mer oligonucleotide is cut out of the gel with a razor blade and eluted either by (1) the crush and soak method of Maxam and Gilbert (PNAS USA 74: 560-568, 1977) or (2) electroelution through an IBI electroelution device. The purified, radiolabeled oligonucleotides are hybridized to the nitrocellulose colony lifts under non-stringent hybridization conditions (37°C for 24 hrs. in 6xSSC, 5 x Denhardt's, 50mM Na-P, pH7.2 and 500 ug/ul of calf thymus DNA). Filters are washed once at room temperature in 6xSSC and 50 mM Na-P, pH 7.2 and again at 37°C in 6xSSC and 50mM Na-P, pH 7.2. Finally, the filters are washed twice at 60°C for 15 minute intervals in 3M tetramethylammonium chloride, 50mM Tris-HCl, ph 7.5, 2mM Na_2-EDTA and 1 mg/ml of SDS. A 21-mer oligonucleotide with a perfect base to base hybridization match (i.e., mutant oligo to mutant phagemid) is not washed free of the phagemid DNA at 60°C, whereas a slight mismatch (i.e., mutant oligo to wild type phagemid) is washed free. Putative positives are streaked from the original LB_{amp} plate to a fresh LB_{amp} plate. Ten transformants from each restreaked putative positive are then restreaked in a grid pattern and the colony hybridization is repeated. Secondary positives are then confirmed via DNA sequence analysis through the Trp574 coding region. (Sanger et al., PNAS VSA 74: 5463-5467, 1977). Two positive mutants, pAC324 (Trp574 -- > Leu substitution) and pAC325 (Trp574 deletion) were selected for further analysis.

EXAMPLE III

Agrobacterium Mediated Transformation of Tobacco

(a) Construction of vectors - Both vectors and the Agrobacterium strain are purchased from Clontech. The plasmid pBIN19 is an E. coli/Agrobacterium binary shuttle vector that possesses both the left and right border sequences of the Ti-plasmid T-DNA, a polylinker, an RK2 bacterial origin of replication functional in both E. coli and Agrobacterium, and a gene conferring resistance to kanamycin (Bevan, Nucl. Acids Res. 12: 8711-8721, 1984) The plasmids pAC324 and pAC325 are digested with XbaI and electrophoresed through a 1% agarose gel. The 5.5Kb XbaI fragment containing the respective mutation of pAC324 and pAC325 are isolated as previously described. The plasmid pBIN19 is digested with XbaI and incubated in a separate ligation reaction with the 5.5Kb XbaI fragment from pAC324 and pAC325. The entire ligation mix is transformed into E. coli XL-1 cells and positive transformants are chosen via a blue/white color selection on LB_{ak} plates (LB plates with 100 ug/ul ampicillin, 80 ug/ml X-Gal and 20mM IPTG). Small scale plasmid preparations, restriction digestion analysis and agarose gel electrophoresis to confirm positive transformants are performed as described previously. Of the four pBIN19 based plasmid constructions, pAC348-pAC349 (both orientations of the 5.5Kb XbaI fragment containing the Trp574-->Leu substitution in pBIN19) and pAC350-pAC351 (both orientations of the 5.5Kb XbaI fragment containing the Trp574 deletion in pBIN19), one construction from each respective mutation is chosen for transformation of tobacco.

(b) Mobilisation of pAC348 - pAC351 into Agrobacterium

The plasmid pRK2013 is a conjugative plasmid that contains trans acting sequences required to mobilize pBIN19 based constructions from E. coli into the disarmed Agrobacterium strain, LBA4404 (pAL4404). This Agrobacterium strain is resistant to streptomycin and contains the disarmed Ti plasmid pAL4404 (Ooms et al., Plasmid 7: 15-29, 1982). This Ti plasmid contributes the transacting virulence functions necessary to facilitate transfer of the pBIN19 based T-DNA region into the chromosome of tobacco. The triparental mating of the pBIN19 based vectors, the conjugative plasmid (pRK2013), and the Agrobacterium strain (LBA4404), are carried out essentially as described by Bevan (Nucl. Acids Res. 12: 8711-8721, 1984. The plasmids pAC348-351 (Kan_r) and pRK2013 (Kan_r) are transformed into competent E. coli HB101 or XL-1 cells as described previously. Transformants containing each plasmid are inoculated into 3ml LB_{kan} broth cultures and incubated at 37°C with constant shaking. The Agrobacterium strain, LBA4404, is inoculated into AB_{strp} media (20 x AB is 20g NH_4Cl, 6g $MgSO_4 \cdot 7H_2O$, 3g KCl, 60g K_2HPO_4,

20g NAH_2PO_4, 3g $CaCl_2$ and 50mg $FeSO_4$-$7H_2O$. Glucose is added to a 1x stock to a final concentration of 0.5x) and incubated with constant shaking at 28°C for 36-48 hr. The triparental mating is initiated by combining 1 ml of each of the three cultures (a pBIN19 based construct, pRK2013 and LBA4404) into a sterile tube and continuing incubation at 28°C for 1 hr. The culture is concentrated via vacuum onto a 0.45uM filter and incubated overnight at 28°C on an NB plate (NB is 4g of Difco nutrient broth powder and 10ml of 1M Tris-HCl, pH 7.2). The filter is placed in 2ml of fresh AB media and rotated slowly for 1 hr. A dilution series is spread onto $AB_{strp/kan}$ plates and incubated at 28°C for 3-4 days. Only Agrobacterium - ($strp_r$) carrying the pBIN19 based construct (kan_r) grow on $AB_{strp/kan}$ media. Single colonies from $AB_{strp/kan}$ plates are inoculated into $AB_{strp/kan}$ broth and incubated at 28°C with constant shaking for 36-48 hr. These cultures are restreaked to $AB_{strp/kan}$ plates, incubated at 28°C for 3-4 days and stored at 4°C until use.

(c) Agrobacterium-Mediated Transformation of Tobacco

pAC348 and pAC351 are inoculated into 50ml of AB_{kan} and incubated with constant shaking at 28°C for 36-48 hr. Bacteria are pelleted by centrifugation at 2500 rpm for 10 minutes in a Damon/IEC table top centrifuge. The bacterial pellet is resuspended in 5ml of BAT media (BAT media is 1x MS salts (Murashige and Skoog, Plant Physiol. 15: 473-497, 1962) 1x B_5 vitamins [100x B_5 is 10mg myo-inositol, 100mg nicotinic acid, 100mg of pyridoxine-HCl and 1000mg Thiamine-HCl per 1 liter], 3% sucrose, 5uM 6-benzylaminopurine at pH 5.7). Young greenhouse grown Wisconsin 38 tobacco leaves are cut into 2 longitudinal sections and soaked in warm water containing Ivory hand soap. The leaf sections are washed in sterile distilled water, soaked in a 10% Clorox + Tween 20 solution for 10 minutes with stirring and rinsed 3x with ddH_2O. Discs are cut from the leaf sections using either a cork borer or hole-punch. These discs are placed in a 50ml tube containing the resuspended Agrobacterium culture. The suspension is mixed gently for 5' minutes and blotted onto a sterile paper towel and cultured onto BAT media plates (100 x 20mm plates with approximately 10 discs per plate). The plates are incubated at 25°C under fluorescent lighting for 48hr. The discs are then transferred to selection media (BATCK; BAT media plus carbenicillin and kanamycin) and returned to the initial incubation conditions until the formation of shoots. Kanamycin resistant shoots are transferred to OTCK (rooting) selection media (OTCK media is BATCK media minus 6-benzylaminopurine) in (GA7) Magenta boxes and the previously described incubation conditions were continued. When kanamycin resistant shoots form an appreciable root system (at least 3 roots with lengths longer than 1cm), the plants are transferred to soil. Briefly, the agar based shoot is removed from the GA7 box, the agar is carefully washed away with warm tap water and the shoots are transferred to metromix in peat pots in GA7 vessels. The plants are placed in the greenhouse and allowed to harden off. A total of 5 kanamycin resistant shoots containing pAC348 and 9 kanamycin resistant shoots containing pAC350 are transferred to soil. Approximately 10-14 days later, the peat pots are transplated to larger pots.

pAC348 Transformant #:	pAC351 Transformant #:
1) 10-1	1) 10-1
2) 33-1	2) 20-1
3) 33-2	3) 20-2
4) 46-1	4) 20-3
5) 46-2	5) 27-1
	6) 27-2
	7) 27-3
	8) 30-1
	9) 42-4

EXAMPLE IV

Determination and Characterization of Mutants Conferring Herbicide Resistance

AHAS enzyme assay (leaf tissue) - Shortly after transfer to the greenhouse, extracts each of the kanamycin resistant transformants are prepared and assayed for insensitivity to the imidazolinone Pursuit® herbicide. Acetohydroxyacid synthase is extracted and assayed as described in the previous section. Two kanamycin resistant transformants (33-1 and 46-2) which contain the Trp574->Leu substitution mutant allele of the Arabidopsis AHAS allele exhibit AHAS activity in the presence of Pursuit® herbicide. In addition,

AHAS activity of one kanamycin resistant deletion transformant (10-1) exhibits insensitivity to the addition of Pursuit® herbicide (Figure 7). This Trp574 deletion transformant is selfed and backcrossed to Wisconsin 38. Progeny from 10-1 exhibit tolerance to post-emergence applications of Pursuit® herbicide 4-8x greater than the concentration lethal to control tobacco plants.

Figures 2(a)-(c) show photographic comparisons of the phenotypes of the crosses and controls discussed above. These observations indicate inheritance of resistance observed in the initial AHAS assay from transformed leaf tissue, and expression of this trait at the whole plant level.

In a more quantitative assay, seed of selfed progeny of the transgenic tobacco plant (10-1) containing the Trp574 deletion mutation of Arabidopsis AHAS as well as seeds of the susceptible parental cultivar "Wisconsin 38" are planted in Metro Mix 350™ in 5" Azalea pots. The seedlings are thinned to the single most vigorous seedling after 2 days. Eleven days later, these plants are sprayed postemergence with Pursuit® at 0, 10, 20, 40, 80 and 160 g/ha. Five plants of each tobacco type (transgenic and control) are sprayed per herbicide rate. Tween 20™ is added at 0.25 v/v to the herbicide solutions prior to spraying. The herbicide is applied with a laboratory belt sprayer at a rate of 400 L/ha at a distance 18" above the plants with a belt speed of 8.2 sec/rev and sprayer nozzle #8501SE. Plant heights are measured 1, 2, 3, and 4 weeks after treatment, plant fresh weight data is collected at four and one-half weeks.

The results of these sprayings are depicted in Figure 4. The progeny data in the graph are presented with the most tolerant individual first and the most susceptible individual last for each herbicide rate. As can be seen from this graph and as would be expected from a selfed progeny of the original transgenic plant, the progeny are segregating for herbicide tolerance. Both susceptible progeny and individuals with varying degrees of herbicide tolerance can be observed.

The plant fresh weights (g) and means for each herbicide rate are presented in Table 2 with the mean fresh weights (g) summarized below (Table 1):

Table 1

Tobacco Mean FW: (5 reps each)	Control	Transgenic Progeny
Herbicide Rate:		
0 g/ha	52.1	53.7
10 g/ha	8.1	36.4
20 g/ha	2.0	28.8
40 g/ha	0.8	20.9
80 g/ha	0.6	26.5
160 g/ha	0.2	21.4

As can been seen in this table, the selfed progeny of the transgenic tobacco plant (10-1) exhibit a high degree of tolerance to Pursuit® applied postemergence. The susceptible parental cultivar does not display herbicide tolerance at any of the rates tested.

Seeds resulting from transgenic plant 10-1 are also assayed for resistance to the imidazolinone Pursuit®. Seeds are plated onto medium containing Pursuit® at 0, 1.25, 2.5, 3.75, and 5.0 µM. Twenty seeds are plated per petri dish, with two dishes per treatment. Herbicide tolerance of seedlings is evaluated after three weeks. The results of the 5.0 µM treatment is presented in Table 2. Even at the lowest concentration (1.25 µM treatment), control plants exhibited herbicide sensitivity. These results illustrate inheritance of the herbicide resistant trait from parental plant 10-1 to its progeny.

Table 2

Results of tobacco seed assay				
Pursuit® Conc.(µM): 5		S*	D	R
Progeny:	W38 (wild-type)	20 20	0 0	0 0
	10-1 Selfed	7 3	6 11	6 6
	10-1 x 0	11 11	9 9	0 0
	0 x 10-1	10 8	9 8	0 0

*S = Susceptible; D=Damaged; R = Resistant

EXAMPLE V

AHAS enzyme assay in E. coli

AHAS enzyme assay (expression in E. coli) - The site directed mutants, pAC224 and pAC225, are utilized as templates for the generation of an E. coli based expression system. The insensitivity to herbicide is tested when the higher plant gene of interest is expressed in a bacterial strain devoid of any endogenous AHAS activity. To this end, the Nco1/Pst1 fragment of wild type Arabidopsis AHAS (pAC301), the Trp574-Leu substitution (pAC224) and the Trp574 deletion (pAC225) are subcloned into the Nco1/Pst1 site of the E. coli expression vector, pKK233-2 (purchased from Pharmacia). This expression plasmid contains an IPTG inducible promoter as well as a transcription termination sequence. Both regions surround an Nco1, HindIII and PstI linker sequence that allows proper orientation of the gene of interest in relation to the bacterial promotor. Subcloning and ligation of the respective NcoI/PstI fragments into NcoI/PstI digested pKK233-2 DNA (ampicillin resistance) is as previously described. The ligation reaction is transformed into the E. coli strain, MF2000 [ilvB800:mu-1, Bg132, ilvI15, thi-1, argE3, RPSL31, (ara-leu, ilvH) 863, mtl-1, xyl-5 galK2, lacYI, recAI]. This bacterial strain is devoid of endogenous AHAS activity. Therefore, growth on a minimal media lacking isoleucine and valine (the end products of amino acid biosynthetic pathway of which the AHAS enzyme is involved) in an MF2000 strain transformed with either pAC224 (Trp574-Leu) or pAC225 (Trp del) requires the expression and function of the Arabidopsis gene in E. coli. Each of the three constructions complements MF2000 on agar media as well as in broth cultures.

Single colonies of MF2000 containing either the plasmid construction pAC210, pAC224 or pAC225 are inoculated into 3ml broth cultures of M63 minimal media (amp) containing the amino acids arginine, leucine, isoleucine and valine. [M63 is 30g KH_2PO_4, 70g K_2HPO_4, 20g $(NH_4)_2SO_4$, 5mg $FeSO_4$, 100ul of 1M $MgSO_4$, 200ul thiamine. The glucose concentration is raised to 1.2%]. The cultures are incubated overnight at 37°C with constant shaking. The bacterial cells are pelleted, resuspended in several mls of M63 (amp) supplemented with arginine and leucine, but lacking isoleucine and valine (minus ilv media). The cells are transferred to 10-50 ml of minus ilv media and constant shaking at 37°C was continued overnight. The cells are pelleted and either used directly in AHAS enzyme assays or frozen at -20°C until use.

The extraction and assay for Arabidopsis AHAS in bacterial cells is essentially as described by Singh, et. al. (J. Chromatography 444: 251-261, 1988). The bacterial pellet is powered in liquid nitrogen and homoganized in 100mM potassium phosphate buffer (pH 7.5) containing 10mM pyruvate, 5mM magnesium chloride, 5mM Na_2-EDTA, 100um flavin adenine dinucleotide (FAD), 1mM valine, 1mM leucine, 10%(v/v) glycerol and 10mM cysteine. The homogenate is filtered through a nylon cloth (53uM mesh) and centrifuged at 25,000 x g for 20 minutes. The supernatant fraction is brought to 50% saturation with respect to ammonium sulfate and allowed to stand for 20-30 minutes on ice. The precipitate is pelleted via centrifugation at 25,000 x g for 20 minutes. The supernatant is discarded and the ammonium sulfate pellet is used immediately or frozen with liquid nitrogen and stored at -20°C until use.

AHAS activity in the presence or absence of herbicide is measured by estimation of the product, acetolactate, after conversion by acid decarboxylation to acetoin. Standard reaction mixtures contain the

enzyme (and herbicide) in 50mM potassium phosphate buffer (pH 7.0) containing 100mM sodium pyruvate, 10mM magnesium chloride, 1mM thiamine pyrophosphate (TPP) and 10uM FAD. This mixture is incubated at 37°C for 1 hr. The reaction is stopped with the addition of sulfuric acid to make a final concentration of 0.85%. The reaction product is allowed to decarboxylate at 60°C for 15 minutes. The acetoin formed is determined by incubating with creatine (0.17%) and 1-naphthol (1.7%) by the method of Westerfeld (J. Biol. Chem. 161: 495-502, 1945). Appropriate checks of direct acetoin formation during the enzyme assays are made.

Wild type Arabidopsis AHAS (pAC201) is sensitive to the imidazolinone herbicide Pursuit® and the sulfonylurea herbicide Glean® whereas both the Trp574-Leu substitution (pAC224) and the Trp574 deletion (pAC225) are insensitive to increasing concentrations of Pursuit® herbicide (Figure 4) and Glean® herbicide (Figure 5). Surprisingly, the deletion confers a level of insensitivity to these herbicides which is equivalent or greater than that of the corresponding substitution. Therefore, deletion of the Trp574 residue of the Arabidopsis AHAS coding region results in a functional form of the enzyme that is insensitive to the addition of either of two unrelated herbicides capable of inhibiting the wild type form of the enzyme.

The procedures noted above are also used to create substitution of an asparagine for a serine at position 653, as well as a deletion at this position. Plasmids containing the substitution (pAC229) or the deletion (pAC230) are transformed into E. coli MF2000 and AHAS activity of deletion and substitution mutants in the presence of herbicides Pursuit® and Glean® is determined for each as described above. The AHAS produced by substitution of asparagine for serine is insensitive to inhibition by Pursuit® (Figure 6) as previously reported (Sathasivan et. al, supra), but sensitivity to Glean® was only slightly reduced (about 10%) as compared with the wild-type enzyme (Figure 7). In contrast, the AHAS produced by the Ser653 deletion is highly resistant to inhibition by both Pursuit® and Glean® (Figures 6 and 7).

DEPOSIT OF BIOLOGICAL MATERIALS

The following microorganisms were deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, 20857, on December 6, 1990, and given accession numbers as follows:

Microorganism	Accession No.
Agrobacterium strain LBA4404 harboring pAC351 (Agro/351) [Trp574 deletion]	68488
Agrobacterium strain LBA4404 harboring plasmid pAC324 [Ser653 deletion]	68489

Sequence ID No.: 1

Sequence Type: Amino Acid

Sequence Length: 670 Amino Acids

Strandedness: Single

Topology: Linear

Original Source Organism: Arabidopsis

Properties: acetohydroxyacid synthase enzyme

```
Met Ala Ala Ala Thr Thr Thr Thr Thr Ser Ser
 1               5                  10

Ser Ile Ser Phe Ser Thr Lys Pro Ser Pro Ser Ser
            15                  20

Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu
 25                  30                  35

Pro Phe Ser Leu Asn Pro Asn Lys Ser Ser Ser Ser
                40                  45

Ser Arg Arg Arg Gly Ile Lys Ser Ser Ser Pro Ser
     50                  55                  60

Ser Ile Ser Ala Val Leu Asn Thr Thr Thr Asn Val
                65                  70

Thr Thr Thr Pro Ser Pro Thr Lys Pro Thr Lys Pro
            75                  80

Glu Thr Phe Ile Ser Arg Leu Ala Pro Asp Gln Pro
 85                  90                  95

Arg Lys Gly Ala Asp Ile Leu Val Glu Ala Leu Glu
                100                 105

Arg Gln Gly Val Glu Thr Val Phe Ala Tyr Pro Gly
    110                 115                 120

Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg
                125                 130

Ser Ser Ser Ile Arg Asn Val Leu Pro Arg His Glu
            135                 140

Gln Gly Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg
145                 150                 155
```

```
Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala Thr Ser
            160                 165

Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala
    170                 175                 180

Asp Ala Leu Leu Asp Ser Val Pro Leu Val Ala Ile
                185                 190

Thr Gly Gln Val Pro Arg Arg Met Ile Gly Thr Asp
        195                 200

Ala Phe Gln Glu Thr Pro Ile Val Glu Val Thr Arg
205                 210                 215

Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val
            220                 225

Glu Asp Ile Pro Arg Ile Ile Glu Glu Ala Phe Phe
    230                 235                 240

Leu Ala Thr Ser Gly Arg Pro Gly Pro Val Leu Val
                245                 250

Asp Val Pro Lys Asp Ile Gln Gln Gln Leu Ala Ile
        255                 260

Pro Asn Trp Glu Gln Ala Met Arg Leu Pro Gly Tyr
265                 270                 275

Met Ser Arg Met Pro Lys Pro Pro Glu Asp Ser His
            280                 285

Leu Glu Gln Ile Val Arg Leu Ile Ser Glu Ser Lys
    290                 295                 300

Lys Pro Val Leu Tyr Val Gly Gly Gly Cys Leu Asn
                305                 310

Ser Ser Asp Glu Leu Gly Arg Phe Val Glu Leu Thr
        315                 320

Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu Gly
325                 330                 335

Ser Tyr Pro Cys Asp Asp Glu Leu Ser Leu His Met
            340                 345

Leu Gly Met His Gly Thr Val Tyr Ala Asn Tyr Ala
    350                 355                 360

Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val
                365                 370
```

Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu Ala
375 380

Phe Ala Ser Arg Ala Lys Ile Val His Ile Asp Ile
385 390 395

Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr Pro His
400 405

Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln
410 415 420

Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu Glu
425 430

Leu Lys Leu Asp Phe Gly Val Trp Arg Asn Glu Leu
435 440

Asn Val Gln Lys Gln Lys Phe Pro Leu Ser Phe Lys
445 450 455

Thr Phe Gly Glu Ala Ile Pro Pro Glu Tyr Ala Ile
460 465

Lys Val Leu Asp Glu Leu Thr Asp Gly Lys Ala Ile
470 475 480

Ile Ser Thr Gly Val Gly Gln His Gln Met Trp Ala
485 490

Ala Gln Phe Tyr Asn Tyr Lys Lys Pro Arg Gln Trp
495 500

Leu Ser Ser Gly Gly Leu Gly Ala Met Gly Phe Gly
505 510 515

Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn Pro
520 525

Asp Ala Ile Val Val Asp Ile Asp Gly Asp Gly Ser
530 535 540

Phe Ile Met Asn Val Gln Glu Leu Ala Thr Ile Arg
545 550

Val Glu Asn Leu Pro Val Lys Val Leu Leu Leu Asn
555 560

Asn Gln His Leu Gly Met Val Met Gln Trp Glu Asp
565 570 575

Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Phe Leu
580 585

Gly Asp Pro Ala Gln Glu Asp Glu Ile Phe Pro Asn
590 595 600

Met Leu Leu Phe Ala Ala Ala Cys Gly Ile Pro Ala
605 610

Ala Arg Val Thr Lys Lys Ala Asp Leu Arg Glu Ala
615 620

Ile Gln Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu
625 630 635

Leu Asp Val Ile Cys Pro His Gln Glu His Val Leu
640 645

Pro Met Ile Pro Ser Gly Gly Thr Phe Asn Asp Val
650 655 660

Ile Thr Glu Gly Asp Gly Arg Ile Lys Tyr
665 670

Sequence ID No.: 2

Sequence Type: Nucleic Acid

Sequence Length: 2,526 Base Pairs

Strandedness: Single

Topology: Linear

Original Source Organism: Arabidopsis

Properties: gene encoding acetohydroxyacid synthase enzyme

NTRAAARAAA GAAAGAAAGA TCAATTTGAT AAATTTCTCA	40
GCCACAAATT CTACATTTAG GTTTTAGCAT ATCGAAGGCT	80
CAATCACAAA TACAATAGAT AGACTAGAGA TTCCAGCGTC	120
ACGTGAGTTT TATCTATAAA TAAAGGACCA AAAATCAAAT	160
CCCGAGGGCA TTTTCGTAAT CCAACATAAA ACCCTTAAAC	200
TTCAAGTCTC ATTTTTAAAC AAATCATGTT CACAAGTCTC	240
TTCTTCTTCT CTGTTTCTCT ATCTCTTGCT CATCTTTCTC	280
CTGAACCATG GCGGCGGCAA CAACAACAAC AACAACATCT	320
TCTTCGATCT CCTTCTCCAC CAAACCATCT CCTTCCTCCT	360
CCAAATCACC ATTACCAATC TCCAGATTCT CCCTCCCATT	400
CTCCCTAAAC CCCAACAAAT CATCCTCCTC CTCCCGCCGC	440
CGCGGTATCA AATCCAGCTC TCCCTCCTCC ATCTCCGCCG	480
TGCTCAACAC AACCACCAAT GTCACAACCA CTCCCTCTCC	520
AACCAAACCT ACCAAACCCG AAACATTCAT CTCCCGATTG	560
GCTCCAGATC AACCCCGCAA AGGCGCTGAT ATCCTCGTCG	600
AAGCTTTAGA ACGTCAAGGC GTAGAAACCG TATTCGCTTA	640
CCCTGGAGGT GCATCAATGG AGATTCACCA AGCCTTAACC	680
CGCTCTTCCT CAATCCGTAA CGTCCTTCCT CGTCACGAAC	720
AAGGAGGTGT ATTCGCAGCA GAAGGATACG CTCGATCCTC	760
AGGTAAACCA GGTATCTGTA TAGCCACTTC AGGTCCCGGA	800

GCTACAAATC TCGTTAGCGG ATTAGCCGAT GCGTTGTTAG 840

ATAGTGTTCC TCTTGTAGCA ATCACAGGAC AAGTCCCTCG 880

TCGTATGATT GGTACAGATG CGTTTCAAGA GACTCCGATT 920

GTTGAGGTAA CGCGTTCGAT TACGAAGCAT AACTATCTTG 960

TGATGGATGT TGAAGATATC CCTAGGATTA TTGAGGAAGC 1000

TTTCTTTTTA GCTACTTCTG GTAGACCTGG ACCTGTTTTG 1040

GTTGATGTTC CTAAAGATAT TCAACAACAG CTTGCGATTC 1080

CTAATTGGGA ACAGGCTATG AGATTACCTG GTTATATGTC 1120

TAGGATGCCT AAACCTCCGG AAGATTCTCA TTTGGAGCAG 1160

ATTGTTAGGT TGATTTCTGA GTCTAAGAAG CCTGTGTTGT 1200

ATGTTGGTGG TGGTTGTTTG AATTCTAGCG ATGAATTGGG 1240

TAGGTTTGTT GAGCTTACGG GGATCCCTGT TGCGAGTACG 1280

TTGATGGGGC TGGGATCTTA TCCTTGTGAT GATGAGTTGT 1320

CGTTACATAT GCTTGGAATG CATGGGACTG TGTATGCAAA 1360

TTACGCTGTG GAGCATAGTG ATTTGTTGTT GGCGTTTGGG 1400

GTAAGGTTTG ATGATCGTGT CACGGGTAAG CTTGAGGCTT 1440

TTGCTAGTAG GGCTAAGATT GTTCATATTG ATATTGACTC 1480

GGCTGAGATT GGGAAGAATA AGACTCCTCA TGTGTCTGTG 1520

TGTGGTGATG TTAAGCTGGC TTTGCAAGGG ATGAATAAGG 1560

TTCTTGAGAA CCGAGCGGAG GAGCTTAAGC TTGATTTTGG 1600

AGTTTGGAGG AATGAGTTGA ACGTACAGAA ACAGAAGTTT 1640

CCGTTGAGCT TTAAGACGTT TGGGGAAGCT ATTCCTCCAC 1680

AGTATGCGAT TAAGGTCCTT GATGAGTTGA CTGATGGAAA 1720

AGCCATAATA AGTACTGGTG TCGGGCAACA TCAAATGTGG 1760

GCGGCGCAGT TCTACAATTA CAAGAAACCA AGGCAGTGGC 1800

TATCATCAGG AGGCCTTGGA GCTATGGGAT TTGGACTTCC 1840

TGCTGCGATT GGAGCGTCTG TTGCTAACCC TGATGCGATA 1880

GTTGTGGATA TTGACGGAGA TGGAAGCTTT ATAATGAATG	1920
TGCAAGAGCT AGCCACTATT CGTGTAGAGA ATCTTCCAGT	1960
GAAGGTACTT TTATTAAACA ACCAGCATCT TGGCATGGTT	2000
ATGCAATGGG AAGATCGGTT CTACAAAGCT AACCGAGCTC	2040
ACACATTTCT CGGGGATCCG GCTCAGGAGG ACGAGATATT	2080
CCCGAACATG TTGCTGTTTG CAGCAGCTTG CGGGATTCCA	2120
GCGGCGAGGG TGACAAAGAA AGCAGATCTC CGAGAAGCTA	2160
TTCAGACAAT GCTGGATACA CCAGGACCTT ACCTGTTGGA	2200
TGTGATTTGT CCGCACCAAG AACATGTGTT GCCGATGATC	2240
CCGAGTGGTG GCACTTTCAA CGATGTCATA ACGGAAGGAG	2280
ATGGCCGGAT TAAATACTGA GAGATGAAAC CGGTGATTAT	2320
CAGAACCTTT TATGGTCTTT GTATGCATAT GGTAAAAAAA	2360
CTTAGTTTGC AATTTCCTGT TTGTTTTGGT AATTTGAGTT	2400
TCTTTTAGTT GTTGATCTGC CTGCTTTTTG GTTTACGTCA	2440
GACTACTACT GCTGTTGTTG TTTGGTTTCC TTTCTTTCAT	2480
TTTATAAATA AATAATCCGG TTCGGTTTAC TCCTTGTGAC	2520
TGGCTC	2526

Claims

1. A method for expressing an herbicide resistant AHAS enzyme in a host cell comprising transforming the host cell with a nucleic acid sequence encoding a functional AHAS enzyme, which enzyme has an amino acid sequence differing from a wild-type AHAS sequence in having at least one amino acid deletion that confers herbicide resistance to the enzyme, and culturing the host cell under conditions permitting expression of the enzyme.

2. The method of Claim 1 in which a deletion is present in a conserved region of the AHAS sequence.

3. The method of any of Claims 1-2 in which a deletion has occurred in a region of the wild type AHAS sequence Arabidopsis selected from the group consisting of amino acids 119-122, 194-197, 201-208, 255-257, 348-353, 373-377, 569-578, and 650-653, or the homologous regions in a different species.

4. The method of Claims 1-3 in which the deletion occurs at at least one position selected from the group consisting of amino acids 121, 122, 197, 205, 256, 351, 376, 571, 574, 578 and 653.

5. The method of any of Claims 1-4 in which resistance is to at least one herbicide selected from the group consisting of an imidazolinone, a sulfonylurea and a triazolopyrimidine.

6. The method of any of Claims 1-5 in which the host cell is a plant cell.

7. The method of Claim 6 in which the transformed plant cell is regenerated to a mature plant expressing an herbicide resistant phenotype.

8. A method for producing a vector useful in conferring herbicide resistance to a host cell comprising ligating into an appropriate vector a nucleic acid sequence encoding a functional AHAS enzyme, which enzyme has an amino acid sequence differing from a wild type AHAS in having at least one amino acid deletion that confers herbicide resistance to the enzyme.

9. A method for conferring herbicide resistance to a plant cell which comprises providing the plant cell with a nucleic acid sequence encoding a functional AHAS enzyme, which enzyme has an amino acid sequence differing from a wild type AHAS sequence in having at least one amino acid deletion that confers herbicide resistance to the enzyme.

10. A method for growing herbicide resistant plants which comprises cultivating a plant which produces a functional AHAS enzyme with an amino acid sequence differing from a wild type AHAS sequence in having at least one amino acid deletion that confers herbicide resistance to the enzyme, in the presense of an inhibitory amount of the herbicide.

11. A method of selecting host cells successfully transformed with a gene of interest which comprises providing to prospective host cells the gene of interest linked to a nucleic acid sequence encoding a functional AHAS enzyme, which enzyme has an amino acid sequence differing from a wild-type AHAS sequence in having at least one amino acid deletion that confers herbicide resistance to the enzyme, and culturing the host cell under conditions permitting expression of the enzyme, growing the cells in the presence of an inhibitory amount of a herbicide, and identifying surviving cells as containing the gene of interest.

```
Met Ala Ala Ala Thr Thr Thr Thr Thr Ser Ser
 1               5                  10

Ser Ile Ser Phe Ser Thr Lys Pro Ser Pro Ser Ser
           15                  20

Ser Lys Ser Pro Leu Pro Ile Ser Arg Phe Ser Leu
 25                  30                  35

Pro Phe Ser Leu Asn Pro Asn Lys Ser Ser Ser Ser
              40                  45

Ser Arg Arg Arg Gly Ile Lys Ser Ser Ser Pro Ser
     50                  55                  60

Ser Ile Ser Ala Val Leu Asn Thr Thr Thr Asn Val
                 65                  70

Thr Thr Thr Pro Ser Pro Thr Lys Pro Thr Lys Pro
           75                  80

Glu Thr Phe Ile Ser Arg Leu Ala Pro Asp Gln Pro
 85                  90                  95

Arg Lys Gly Ala Asp Ile Leu Val Glu Ala Leu Glu
              100                 105

Arg Gln Gly Val Glu Thr Val Phe Ala Tyr Pro Gly
     110                 115                 120

Gly Ala Ser Met Glu Ile His Gln Ala Leu Thr Arg
              125                 130

Ser Ser Ser Ile Arg Asn Val Leu Pro Arg His Glu
           135                 140

Gln Gly Gly Val Phe Ala Ala Glu Gly Tyr Ala Arg
145                 150                 155

Ser Ser Gly Lys Pro Gly Ile Cys Ile Ala Thr Ser
              160                 165

Gly Pro Gly Ala Thr Asn Leu Val Ser Gly Leu Ala
     170                 175                 180

Asp Ala Leu Leu Asp Ser Val Pro Leu Val Ala Ile
              185                 190

Thr Gly Gln Val Pro Arg Arg Met Ile Gly Thr Asp
           195                 200

Ala Phe Gln Glu Thr Pro Ile Val Glu Val Thr Arg
205                 210                 215
```

FIG.1a(i)

Ser Ile Thr Lys His Asn Tyr Leu Val Met Asp Val
220 225

Glu Asp Ile Pro Arg Ile Ile Glu Glu Ala Phe Phe
230 235 240

Leu Ala Thr Ser Gly Arg Pro Gly Pro Val Leu Val
245 250

Asp Val Pro Lys Asp Ile Gln Gln Gln Leu Ala Ile
255 260

Pro Asn Trp Glu Gln Ala Met Arg Leu Pro Gly Tyr
265 270 275

Met Ser Arg Met Pro Lys Pro Pro Glu Asp Ser His
280 285

Leu Glu Gln Ile Val Arg Leu Ile Ser Glu Ser Lys
290 295 300

Lys Pro Val Leu Tyr Val Gly Gly Gly Cys Leu Asn
305 310

Ser Ser Asp Glu Leu Gly Arg Phe Val Glu Leu Thr
315 320

Gly Ile Pro Val Ala Ser Thr Leu Met Gly Leu Gly
325 330 335

Ser Tyr Pro Cys Asp Asp Glu Leu Ser Leu His Met
340 345

Leu Gly Met His Gly Thr Val Tyr Ala Asn Tyr Ala
350 355 360

Val Glu His Ser Asp Leu Leu Leu Ala Phe Gly Val
365 370

Arg Phe Asp Asp Arg Val Thr Gly Lys Leu Glu Ala
375 380

Phe Ala Ser Arg Ala Lys Ile Val His Ile Asp Ile
385 390 395

Asp Ser Ala Glu Ile Gly Lys Asn Lys Thr Pro His
400 405

Val Ser Val Cys Gly Asp Val Lys Leu Ala Leu Gln
410 415 420

Gly Met Asn Lys Val Leu Glu Asn Arg Ala Glu Glu
425 430

FIG.1a(ii)

```
Leu Lys Leu Asp Phe Gly Val Trp Arg Asn Glu Leu
          435                 440

Asn Val Gln Lys Gln Lys Phe Pro Leu Ser Phe Lys
445                 450                 455

Thr Phe Gly Glu Ala Ile Pro Pro Gln Tyr Ala Ile
               460                 465

Lys Val Leu Asp Glu Leu Thr Asp Gly Lys Ala Ile
    470                 475                 480

Ile Ser Thr Gly Val Gly Gln His Gln Met Trp Ala
                    485                 490

Ala Gln Phe Tyr Asn Tyr Lys Lys Pro Arg Gln Trp
          495                 500

Leu Ser Ser Gly Gly Leu Gly Ala Met Gly Phe Gly
505                 510                 515

Leu Pro Ala Ala Ile Gly Ala Ser Val Ala Asn Pro
               520                 525

Asp Ala Ile Val Val Asp Ile Asp Gly Asp Gly Ser
    530                 535                 540

Phe Ile Met Asn Val Gln Glu Leu Ala Thr Ile Arg
                    545                 550

Val Glu Asn Leu Pro Val Lys Val Leu Leu Leu Asn
          555                 560

Asn Gln His Leu Gly Met Val Met Gln Trp Glu Asp
565                 570                 575

Arg Phe Tyr Lys Ala Asn Arg Ala His Thr Phe Leu
               580                 585

Gly Asp Pro Ala Gln Glu Asp Glu Ile Phe Pro Asn
    590                 595                 600

Met Leu Leu Phe Ala Ala Ala Cys Gly Ile Pro Ala
                    605                 610

Ala Arg Val Thr Lys Lys Ala Asp Leu Arg Glu Ala
          615                 620

Ile Gln Thr Met Leu Asp Thr Pro Gly Pro Tyr Leu
625                 630                 635

Leu Asp Val Ile Cys Pro His Gln Glu His Val Leu
               640                 645
```

FIG.1a(iii)

```
Pro Met Ile Pro Ser Gly Gly Thr Phe Asn Asp Val
   650                 655                 660

Ile Thr Glu Gly Asp Gly Arg Ile Lys Tyr
                665                 670
```

FIG.1a(iiii)

```
NTRAAARAAA GAAAGAAAGA TCAATTTGAT AAATTTCTCA      40
GCCACAAATT CTACATTTAG GTTTTAGCAT ATCGAAGGCT      80
CAATCACAAA TACAATAGAT AGACTAGAGA TTCCAGCGTC     120
ACGTGAGTTT TATCTATAAA TAAAGGACCA AAAATCAAAT     160
CCCGAGGGCA TTTTCGTAAT CCAACATAAA ACCCTTAAAC     200
TTCAAGTCTC ATTTTTAAAC AAATCATGTT CACAAGTCTC     240
TTCTTCTTCT CTGTTTCTCT ATCTCTTGCT CATCTTTCTC     280
CTGAACCATG GCGGCGGCAA CAACAACAAC AACAACATCT     320
TCTTCGATCT CCTTCTCCAC CAAACCATCT CCTTCCTCCT     360
CCAAATCACC ATTACCAATC TCCAGATTCT CCCTCCCATT     400
CTCCCTAAAC CCCAACAAAT CATCCTCCTC CTCCCGCCGC     440
CGCGGTATCA AATCCAGCTC TCCCTCCTCC ATCTCCGCCG     480
TGCTCAACAC AACCACCAAT GTCACAACCA CTCCCTCTCC     520
AACCAAACCT ACCAAACCCG AAACATTCAT CTCCCGATTG     560
GCTCCAGATC AACCCCGCAA AGGCGCTGAT ATCCTCGTCG     600
AAGCTTTAGA ACGTCAAGGC GTAGAAACCG TATTCGCTTA     640
CCCTGGAGGT GCATCAATGG AGATTCACCA AGCCTTAACC     680
CGCTCTTCCT CAATCCGTAA CGTCCTTCCT CGTCACGAAC     720
AAGGAGGTGT ATTCGCAGCA GAAGGATACG CTCGATCCTC     760
AGGTAAACCA GGTATCTGTA TAGCCACTTC AGGTCCCGGA     800
GCTACAAATC TCGTTAGCGG ATTAGCCGAT GCGTTGTTAG     840
ATAGTGTTCC TCTTGTAGCA ATCACAGGAC AAGTCCCTCG     880
TCGTATGATT GCTACAGATG CGTTTCAAGA GACTCCGATT     920
GTTGAGGTAA CGCGTTCGAT TACGAAGCAT AACTATCTTG     960
TGATGGATGT TGAAGATATC CCTAGGATTA TTGAGGAAGC    1000
TTTCTTTTTA GCTACTTCTG GTAGACCTGG ACCTGTTTTG    1040
GTTGATGTTC CTAAAGATAT TCAACAACAG CTTGCGATTC    1080
```

FIG.1b(i)

```
CTAATTGGGA ACAGGCTATG AGATTACCTG GTTATATGTC   1120
TAGGATGCCT AAACCTCCGG AAGATTCTCA TTTGGAGCAG   1160
ATTGTTAGGT TGATTTCTGA GTCTAAGAAG CCTGTGTTGT   1200
ATGTTGGTGG TGGTTGTTTG AATTCTAGCG ATGAATTGGG   1240
TAGGTTTGTT GAGCTTACGG GGATCCCTGT TGCGAGTACG   1280
TTGATGGGGC TGGGATCTTA TCCTTGTGAT GATGAGTTGT   1320
CGTTACATAT GCTTGGAATG CATGGGACTG TGTATGCAAA   1360
TTACGCTGTG GAGCATAGTG ATTTGTTGTT GGCGTTTGGG   1400
GTAAGGTTTG ATGATCGTGT CACGGGTAAG CTTGAGGCTT   1440
TTGCTAGTAG GGCTAAGATT GTTCATATTG ATATTGACTC   1480
GGCTGAGATT GGGAAGAATA AGACTCCTCA TGTGTCTGTG   1520
TGTGGTGATG TTAAGCTGGC TTTGCAAGGG ATGAATAAGG   1560
TTCTTGAGAA CCGAGCGGAG GAGCTTAAGC TTGATTTTGG   1600
AGTTTGGAGG AATGAGTTGA ACGTACAGAA ACAGAAGTTT   1640
CCGTTGAGCT TTAAGACGTT TGGGGAAGCT ATTCCTCCAC   1680
AGTATGCGAT TAAGGTCCTT GATGAGTTGA CTGATGGAAA   1720
AGCCATAATA AGTACTGGTG TCGGGCAACA TCAAATGTGG   1760
GCGGCGCAGT TCTACAATTA CAAGAAACCA AGGCAGTGGC   1800
TATCATCAGG AGGCCTTGGA GCTATGGGAT TTGGACTTCC   1840
TGCTGCGATT GGAGCGTCTG TTGCTAACCC TGATGCGATA   1880
GTTGTGGATA TTGACGGAGA TGGAAGCTTT ATAATGAATG   1920
TGCAAGAGCT AGCCACTATT CGTGTAGAGA ATCTTCCAGT   1960
GAAGGTACTT TTATTAAACA ACCAGCATCT TGGCATGCTT   2000
ATGCAATGGG AAGATCGGTT CTACAAAGCT AACCGAGCTC   2040
ACACATTTCT CGGGGATCCG GCTCAGGAGG ACGAGATATT   2080
CCCGAACATG TTGCTGTTTG CAGCAGCTTG CGGGATTCCA   2120
GCGGCGAGGG TGACAAAGAA AGCAGATCTC CGAGAAGCTA   2160
```

FIG.1b(ii)

TTCAGACAAT GCTGGATACA CCAGGACCTT ACCTGTTGGA	2200
TGTGATTTGT CCGCACCAAG AACATGTGTT GCCGATGATC	2240
CCGAGTGGTG GCACTTTCAA CGATGTCATA ACGGAAGGAG	2280
ATGGCCGGAT TAAATACTGA GAGATGAAAC CGGTGATTAT	2320
CAGAACCTTT TATGGTCTTT GTATGCATAT GGTAAAAAAA	2360
CTTAGTTTGC AATTTCCTGT TTGTTTTGGT AATTTGAGTT	2400
TCTTTTAGTT GTTGATCTGC CTGCTTTTTG GTTTACGTCA	2440
GACTACTACT GCTGTTGTTG TTTGGTTTCC TTTCTTTCAT	2480
TTTATAAATA AATAATCCGG TTCGGTTTAC TCCTTGTGAC	2520
TGGCTC	2526

FIG.1b(iii)

FIG.2a

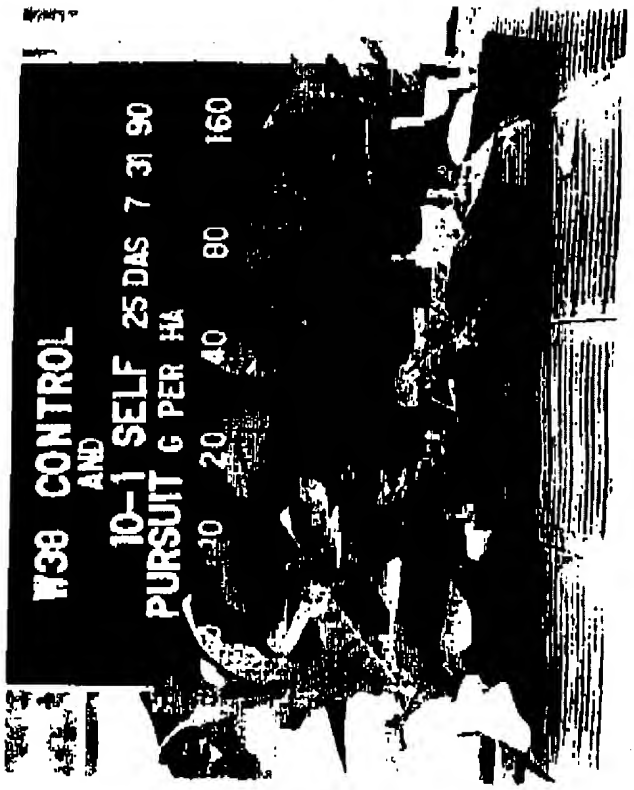
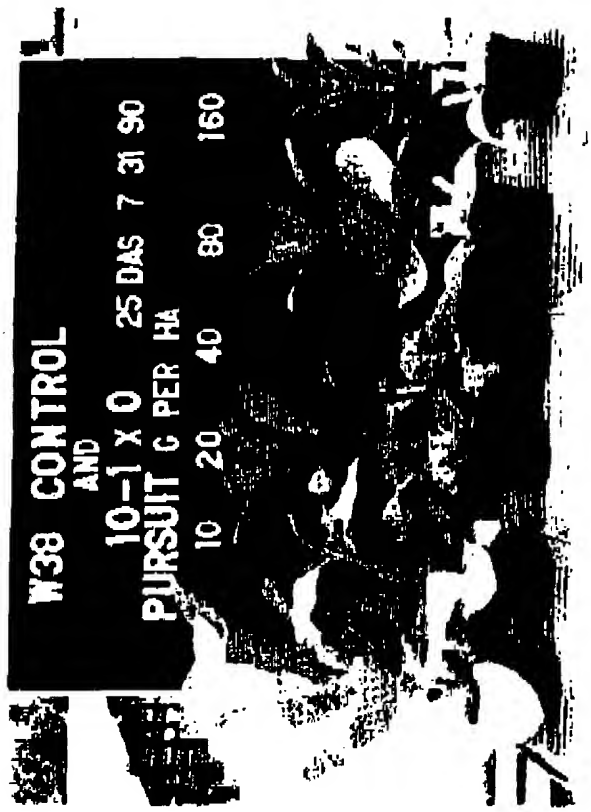

W38 CONTROL
AND
10-1 SELF 25 DAS 7 31 90
PURSUIT G PER HA
0
20
40
80
160

FIG. 2b

FIG.2c


W38 CONTROL
AND
10-1 X O
PURSUIT C PER HA
10 20 40 80 160
25 DAS 7 31 90

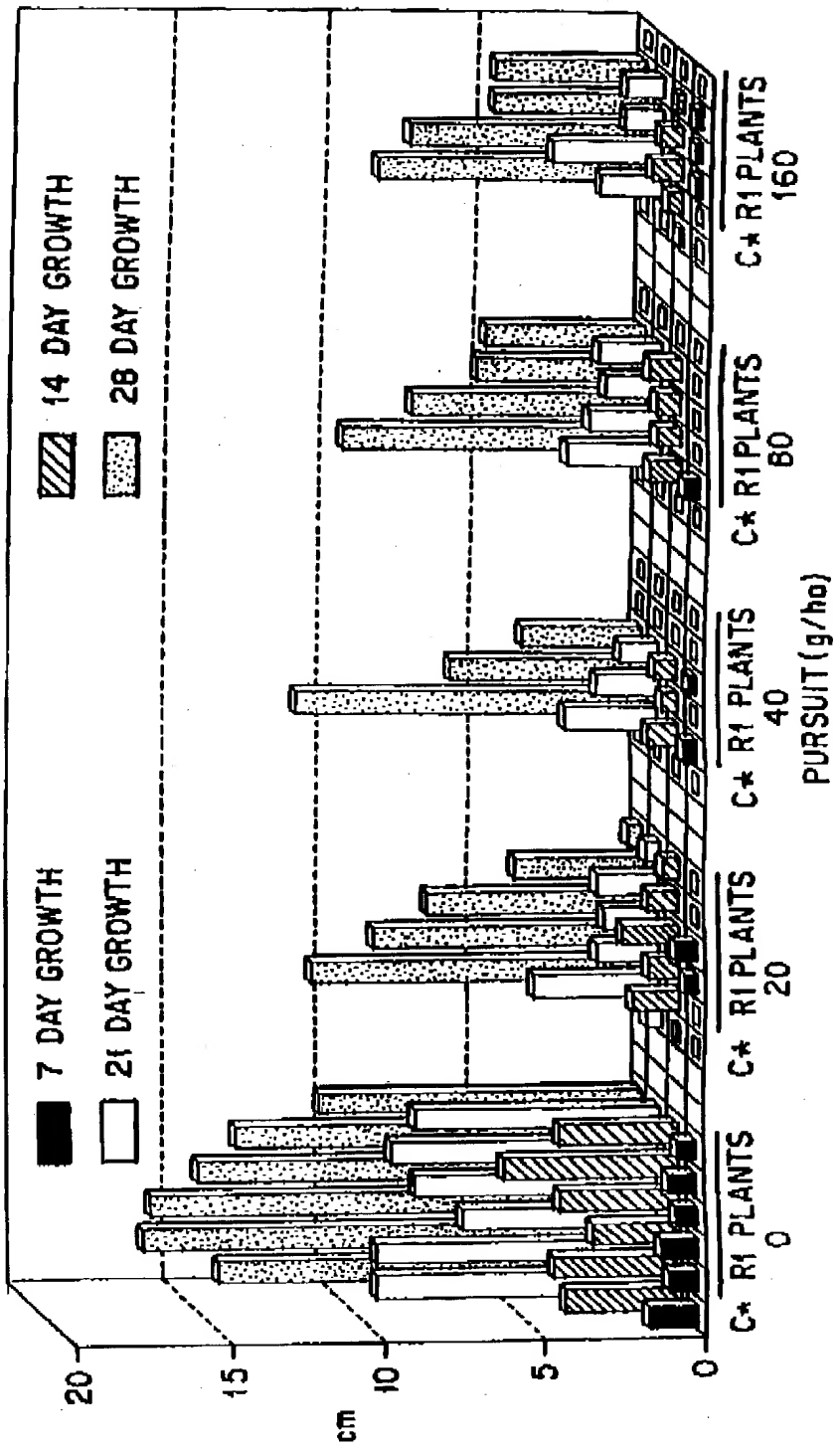

7 DAY GROWTH
14 DAY GROWTH
21 DAY GROWTH
28 DAY GROWTH
C* R1 PLANTS
0
C* R1 PLANTS
20
C* R1 PLANTS
40
C* R1 PLANTS
80
C* R1 PLANTS
160
PURSUIT (g/ha)
0
5
10
15
20
cm

FIG. 3

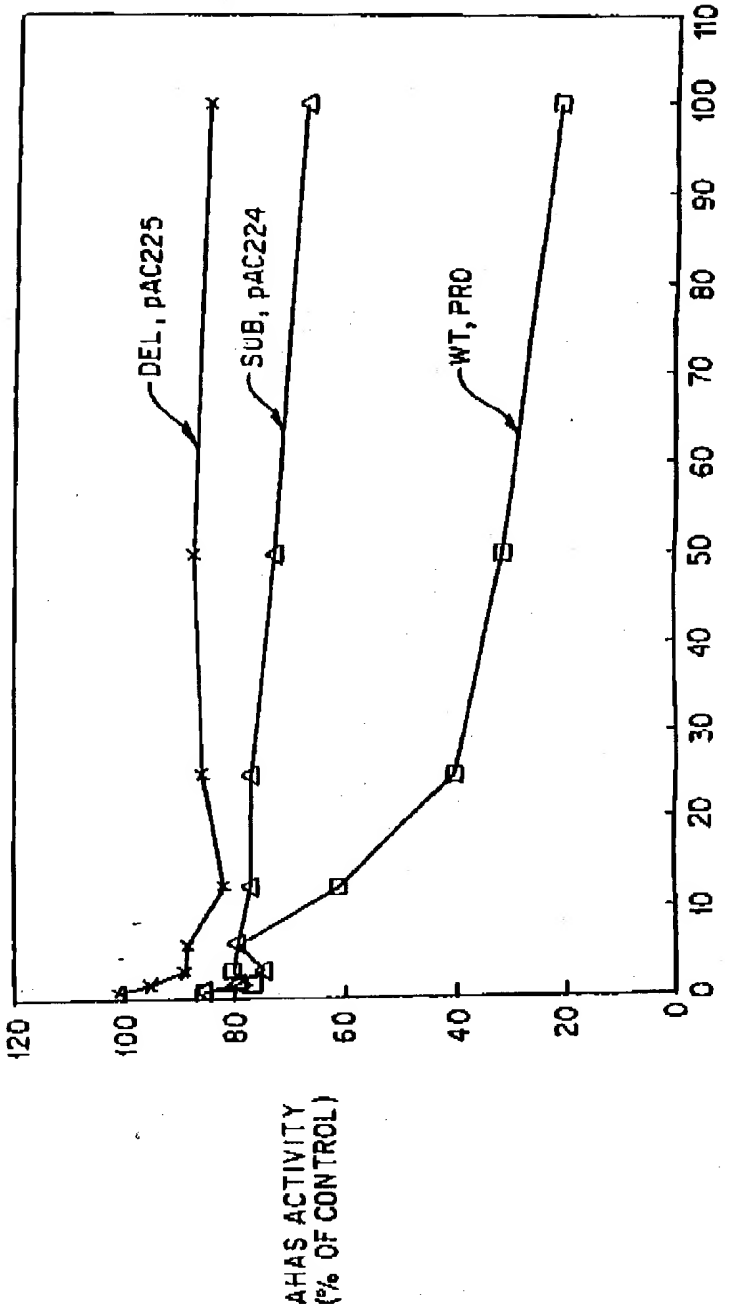

DEL, pAC225
SUB, pAC224
WT, PRO
AHAS ACTIVITY (% OF CONTROL)
[PURSUIT], μM
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
110

FIG. 4

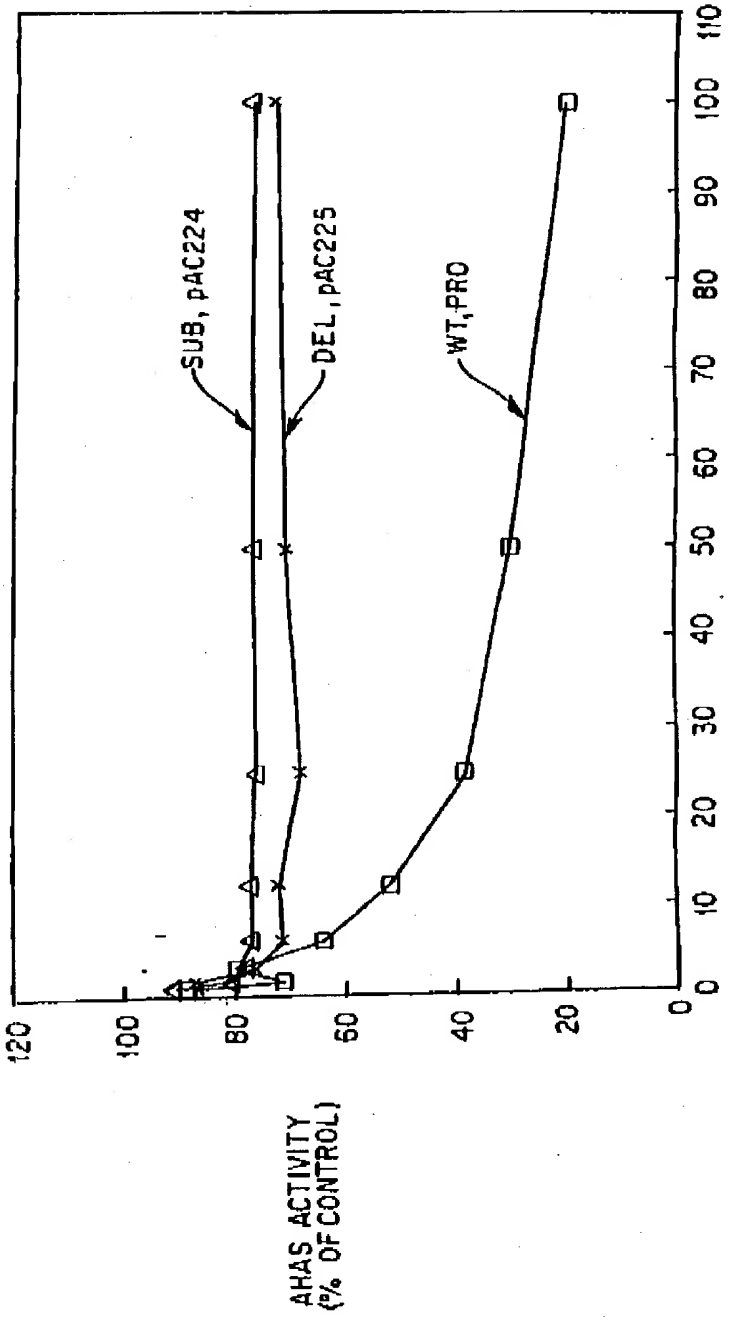


FIG. 5

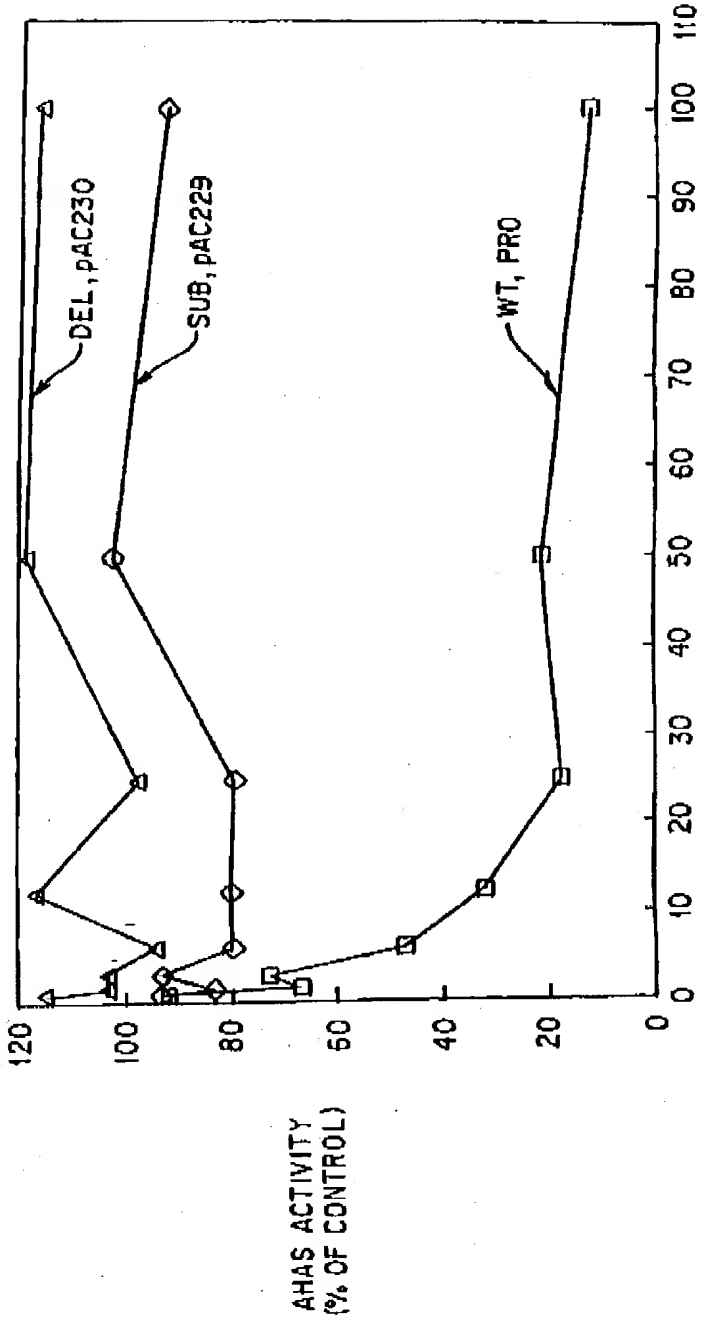

DEL, pAC230
SUB, pAC229
WT, PRO
AHAS ACTIVITY (% OF CONTROL)
[PURSUIT], μM
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100
110

FIG. 6

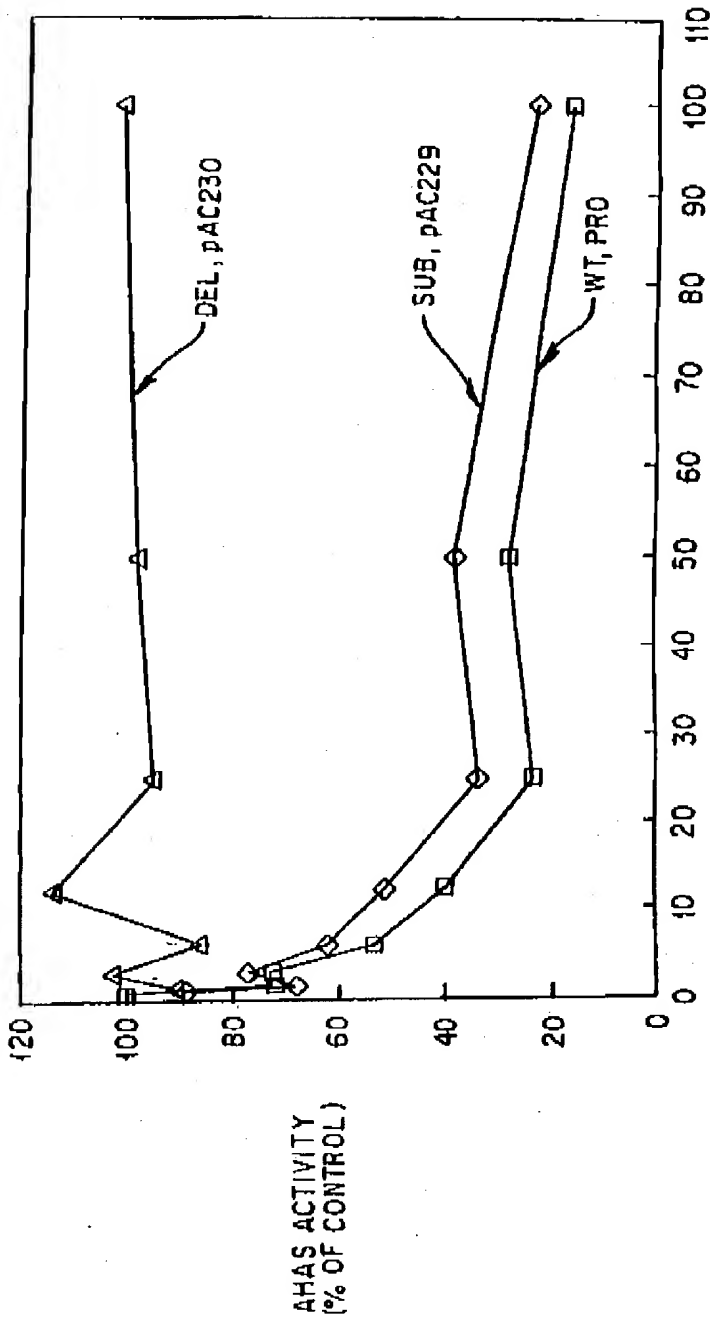


FIG. 7

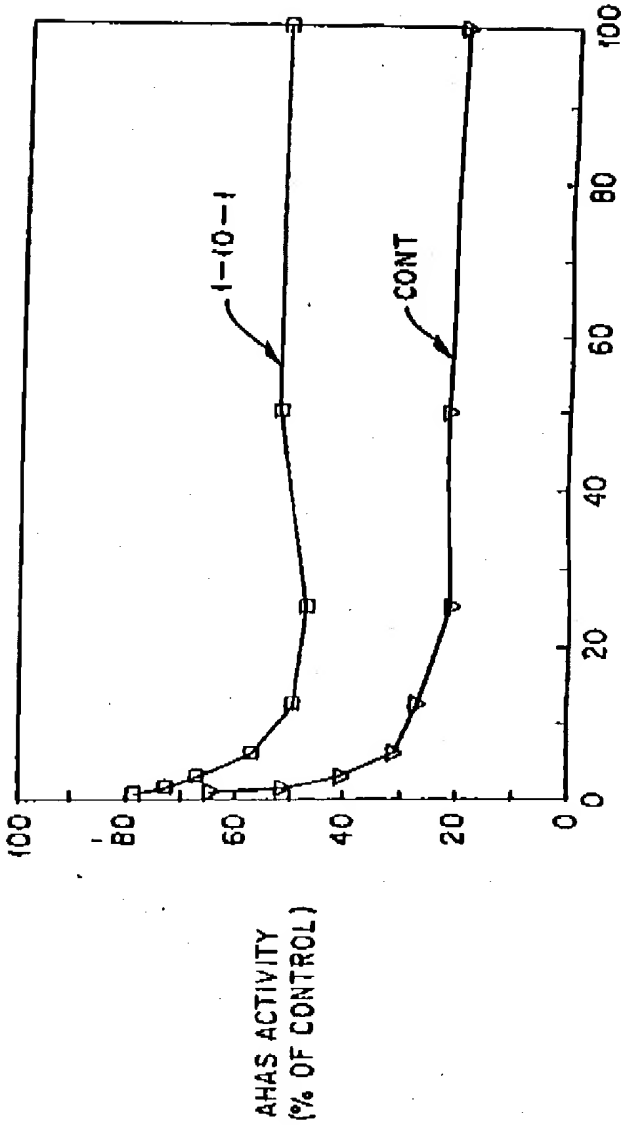


FIG. 8